

Research

Demographic stochasticity alters expected outcomes in experimental and simulated non-neutral communities

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Oikos

128: 1704–1715, 2019

doi: 10.1111/oik.06028

Subject Editor: François Massol

Editor-in-Chief: Dries Bonte

Accepted 25 June 2019

Theory has shown that the effects of demographic stochasticity on communities may depend on the magnitude of fitness differences between species. In particular, it has been suggested that demographic stochasticity has the potential to significantly alter competitive outcomes when fitness differences are small (nearly neutral), but that it has negligible effects when fitness differences are large (highly non-neutral). Here we test such theory experimentally and extend it to examine how demographic stochasticity affects exclusion frequency and mean densities of consumers in simple, but non-neutral, consumer–resource communities. We used experimental microcosms of protists and rotifers feeding on a bacterial resource to test how varying absolute population sizes (a driver of demographic stochasticity) affected the probability of competitive exclusion of the weakest competitor. To explore whether demographic stochasticity could explain our experimental results, and to generalize beyond our experiment, we paired the experiment with a continuous-time stochastic model of resource competition, which we simulated for 11 different fitness inequalities between competing consumers. Consistent with theory, in both our experiments and our simulations we found that demographic stochasticity altered competitive outcomes in communities where fitness differences were small. However, we also found that demographic stochasticity alone could affect communities in other ways, even when fitness differences between competitors were large. Specifically, demographic stochasticity altered mean densities of both weak and strong competitors in experimental and simulated communities. These findings highlight how demographic stochasticity can change both competitive outcomes in non-neutral communities and the processes underlying overall community dynamics.

Keywords: community, competition, demographic stochasticity

Introduction

Studies of interspecific competition in communities often treat competition as a deterministic process, meaning that the same conditions (e.g. genes, traits, environment, etc.) are expected to produce the same competitive outcomes (e.g. coexistence, exclusion). For example, it is common to describe communities using deterministic

equations representing population sizes or densities (e.g. Lotka–Volterra equations, Lotka 1925, Volterra 1926) and use their solutions to predict whether species will coexist in different environments (Tilman 1982, Chesson 2000). However, an important limitation of these approaches is that they are generally unable to account for the variable competitive outcomes frequently observed in real communities (Park 1954, Dickerson and Robinson 1985, Fox and Smith 1997, Fukami 2004).

One reason deterministic approaches can fail is that communities are affected by demographic stochasticity, the randomness in population demographic rates (e.g. birth, death) arising from the (effectively) probabilistic nature of biological processes (May 1973, Renshaw 1991, Melbourne 2012). The importance of demographic stochasticity for populations is well-established, with a large body of theoretical and experimental work showing that it significantly increases extinction risk for small populations (Bartlett 1960, Kurtz 1970, Shaffer 1981, Lande 1993, Burkey 1997, Belovsky et al. 1999, Matis and Kiffe 2000, Griffen and Drake 2008, Ovaskainen and Meerson 2010). However, generalizing beyond these effects on extinction risk has been difficult, insofar as the impacts of demographic stochasticity appear to be context-specific, depending on factors such as population structure (Engen et al. 2005), vital rates (Vindenes et al. 2008), and non-linearities of demographic processes (Bolnick et al. 2011). Further, even though demographic stochasticity tends to be most important in small populations, large populations may also be strongly affected by demographic stochasticity if total abundance is divided across many different life stages or if particular life stages play major roles in demography (Melbourne and Hastings 2008). For communities composed of multiple species subject to these effects, it is even less clear how and under what circumstances demographic stochasticity can influence species abundances.

Most recent studies of the impacts of demographic stochasticity at the community level have considered its influence only with respect to neutral theory (Bell 2000, Hubbell 2001). If a community is neutral such that species fitnesses are identical, demographic stochasticity is expected to be the primary driver of compositional variation. Thus, neutral models incorporating demographic stochasticity and no fitness differences between species are frequently applied to empirical datasets to determine whether they can reproduce observed community patterns (Volkov et al. 2003, Chave 2004, Tilman 2004, Adler et al. 2007, Chase and Myers 2011, Rosindell et al. 2012, Vellend et al. 2014, Wang et al. 2016). However, while the importance of demographic stochasticity in neutral communities is well recognized, there is little appreciation for the idea that it can have effects on communities that are not neutral.

The small number of theoretical and simulation studies on the role of demographic stochasticity in non-neutral communities have largely focused on how it affects coexistence, showing a range of impacts, including both higher

and lower probabilities of coexistence (Orrock and Fletcher 2005, Orrock and Watling 2010, Kramer and Drake 2014, Capitán et al. 2015, Okuyama 2015, Pedruski et al. 2015). Several theoretical analyses suggest that the importance of stochasticity for coexistence appears to depend on the magnitude of fitness differences between co-occurring species (Orrock and Fletcher 2005, Orrock and Watling 2010, Pedruski et al. 2015). However, this has not been tested empirically. Indeed, much of the empirical work related to demographic stochasticity in non-neutral communities has tended to proceed independent of theory, focusing on its effects on long-term community-level patterns such as species diversity (Spencer and Warren 1996, Fukami 2004, Wang et al. 2016), or on factors only associated with coexistence such as mean growth rate (Siepielski et al. 2010, Gilbert and Levine 2017). Of the few studies that do consider finer-scale outcomes such as abundances over time, most examine the effects of demographic stochasticity alongside other factors such as dispersal (Alexander et al. 2012), priority effects (Mertz et al. 1976, Fukami 2004) or density manipulations (Svensson et al. 2018). These approaches are incomplete because even with fine experimental control, the effects of demographic stochasticity are difficult to isolate from other drivers of variability without ample time-series data (Vellend et al. 2014, Engen et al. 2017), and the application of detailed stochastic population models (Melbourne and Hastings 2008).

Here we focus on testing the effects of demographic stochasticity in simple non-neutral, two-species communities, using both replicated experimental microcosms and stochastic simulations of communities in continuous-time. We examine how stochasticity affects non-neutral community dynamics, in particular testing how it affects the probability of competitive exclusion and mean densities over time. Our approach considers two sets of analyses:

- 1) Experimental microcosms. We created three types of two-species communities, consisting of protist and rotifer competitors with a range of competitive abilities between them (i.e. from small to large differences in relative fitness). We manipulated the strength of demographic stochasticity in these communities by varying absolute abundances over two orders of magnitude (500–40 000 individuals). We then observed how our manipulations affected competitive outcomes and species densities over time.
- 2) Stochastic simulations. We used Gillespie's stochastic simulation algorithm (SSA) to simulate a consumer–resource model for a range of absolute abundances and differences in competitive abilities, similar to those in our experiment. We then observed how absolute abundance affected competitive dynamics. Any changes to competitive outcomes or mean densities in these simulations would reflect real, practical effects that demographic stochasticity alone could have on non-neutral communities such as ours.

Material and methods

Part 1. Experimental microcosms

We used laboratory microcosms to examine the impact of absolute abundance (a proxy for demographic stochasticity) on competitive outcomes and dynamics. Experimental units consisted of different sized jars containing liquid medium, bacteria and one or two protist/rotifer species. The three species used were the ciliate protists *Paramecium aurelia* and *Paramecium caudatum*, and the bdelloid rotifer *Philodina americanum*. All three species are fast-growing bacterivores and came from large laboratory stock cultures (>1.5 years old) raised under constant conditions. We knew from 1.5 years of maintaining stock cultures of the three species that the competitive hierarchy in our system was as follows: *Philodina americanum* >> *P. caudatum* > *P. aurelia*. This competitive hierarchy is also described in Cadotte et al. (2006), where for similar conditions *P. americanum* was the superior competitor and *P. aurelia* and *P. caudatum* had overlapping competitive ranks.

Microcosm medium was spring water collected from Big Hill Springs Provincial Park, Alberta (Canada), mixed with 1.0 g l^{-1} of crushed protozoan pellets. Prior to the start of the experiment, the medium was autoclaved and allowed to cool for 1.5 h before being vacuum-filtered through autoclaved Whatman GF/A filters (two–three for each one of fluid) to remove large particles. Each litre of filtrate was then inoculated with a long-running (>1.5 years) lab strain of unidentified bacteria originally isolated from a stock culture of *Colpidium striatum*. All filtrations and inoculations were done under sterile conditions in a laminar flow hood, but it is likely that small amounts of unknown bacteria were also introduced into the medium at this time. The inoculated medium was then loosely capped and stored in an incubator with constant light and a constant temperature of 22°C (stirred every 10–12 h) until day 0 of the experiment (66 h total).

Three microcosm sizes were used to manipulate the maximum population size of the protist communities: 1 ml (small), 10 ml (medium) and 80 ml (large). Air surface-to-volume ratios were constant between small and large jars, but were slightly lower in medium-sized jars. Except during sampling, all jars were kept with loose caps in incubators with constant temperature (22°C) and 24 h of light for the duration of the experiment.

On day 0 of the experiment (12 August 2013), autoclaved jars were filled in a laminar flow hood with the inoculated medium and seeded with all possible two-species combinations of the three microzooplankton species: *P. aurelia* and *P. caudatum* (*aurelia*–*caudatum*), *P. aurelia* and *P. americanum* (*aurelia*–*philodina*) and *P. americanum* and *P. caudatum* (*philodina*–*caudatum*).

In the small jars, five individuals of each relevant species were added directly to the jars to create initial densities of exactly 5 ml^{-1} . For the medium and large jars, it was impractical to add a precise number of individuals to attain

starting densities of 5 ml^{-1} , so we subsampled individuals from stock cultures of known densities to create initial densities of approximately five individuals ml^{-1} . Each combination of jar size and species pairing was replicated 12 times, for a total of 108 experimental microcosms. To confirm the results of previous experiments that have shown that these species compete (Gause 1934, Vandermeer 1969, Cadotte et al. 2006, DeLong and Vasseur 2012), we also established 10 replicate single-consumer cultures of each species at the large jar size and report those results in Supplementary material Appendix 1.

Sampling and census

On day two and every two days thereafter, jars were shaken to homogenize the distribution of microzooplankton and a 0.3 ml subsample was removed. Subsamples were carefully scanned under a dissecting microscope to count the number of individuals of each species. Then, subsamples were returned to the jars (i.e. non-destructive sampling). In practice, a small amount of liquid (free of protists and/or rotifers) was left behind on the counting plates, meaning that our sampling procedure gradually reduced the volume of liquid within jars over time. This effect was only noticeable in the small jars beginning after day 36.

On day eight and every eight days thereafter, 9% of the fluid (including protists and/or rotifers) in each jar (0.09 ml for small jars, 0.9 ml for medium jars, 7.2 ml for large jars) was removed in a laminar flow hood and replaced with new sterile media (made as above, except without the addition of bacteria) at a volume equal to 10% of the nominal culture volume (0.1 ml for small jars, 1 ml for medium jars, 8 ml for large jars). We replaced a larger volume of medium than we removed to account for evaporation and medium lost during sampling.

Individual jars were removed from the experiment once competitive exclusion had been observed. Competitive exclusion was defined as the absence of one of the two competing species in the 0.3 ml subsample and, for the medium and large jars, from an additional 10% subsample (1 ml for medium jars, 8 ml for large jars). If species were not found in the first sample but could be seen in the 10% subsample, we instead used the 10% subsample to estimate the patch density on that day and the jar was kept in the experiment. We ended the experiment on day 72 after observing the last exclusion in our competitive pairings.

Analysis of competitive outcomes and mean densities

To assess the effects of demographic stochasticity on competitive outcomes, we compared exclusion frequency for the *aurelia*–*caudatum*, *aurelia*–*philodina* and *philodina*–*caudatum* pairings across jar sizes. We tested the effect of jar size on competitive outcome statistically using a logit model (family: binomial; exclusion by the superior competitor was scored as 1, 0 otherwise), with species pairing and jar size as

categorical explanatory variables. All jars had the same initial and maximum densities, differing only in their initial and maximum absolute abundances. In addition, environmental conditions and initial resources were the same for jars of all sizes. Thus, jar size was treated as a proxy for the strength of demographic stochasticity.

We examined the effects of jar size on densities over time by comparing the 95% confidence intervals of mean densities in different jar sizes. We focus on estimating and reporting experimental means and their uncertainties but note that if the 95% confidence intervals of two means do not overlap, the difference between the means is generally considered significant. Using confidence intervals of densities over time allowed us to broadly (and conservatively) compare population trajectories in the different jar sizes.

Part 2. Stochastic simulations of consumer–resource dynamics

We used simulations of a simple stochastic consumer–resource model to understand how demographic stochasticity could alter competitive outcomes and mean densities over time in two-consumer, non-neutral communities. The model describes two consumers (N_1 , N_2) competing for a shared resource (R), the deterministic version of which is as follows (MacArthur 1970):

$$\frac{dR}{dt} = rR \left(1 - \frac{R}{K} \right) - a_1 R N_1 - a_2 R N_1 \quad (1)$$

$$\frac{dN_1}{dt} = e_1 a_1 R N_1 - d_1 N_1$$

$$\frac{dN_2}{dt} = e_2 a_2 R N_2 - d_1 N_1$$

where r is the density-independent growth rate (i.e. births minus deaths) of the resource, K is the carrying capacity of the resource, a_i is the area of attack of consumer i (proportional to the size of the system), e_i is the conversion efficiency of consumer i , and d_i is the density-independent per capita mortality rate of consumer i .

When only a single consumer is present in model 1, the resource R has a stable equilibrium (R^*) equal to $d_i/e_i a_i$. This equilibrium is the lowest abundance/density of the resource at which consumer i has a non-negative growth rate. In the two-consumer case, the system will approach or oscillate towards the lowest of these R^* 's, meaning that the consumer with the lowest R^* will ultimately exclude its competitor (Tilman 1982). Differences in consumers' R^* are known as fitness inequalities in ecology (Chesson 2000, Adler et al. 2007), which we describe using the formula (Pedruski et al. 2015):

$$\frac{R_{N_1}^*}{R_{N_2}^*} - \frac{R_{N_2}^*}{R_{N_1}^*} \quad (2)$$

where $R_{N_i}^*$ is the stable equilibrium for the resource when only N_i is present. Using the above formula, the fitness inequality is 0 when consumers have identical R^* 's and is positive when $R_{N_1}^* > R_{N_2}^*$ (i.e. when consumer N_1 requires more of the resource to persist).

An analogous stochastic version of model 1 can be derived by assigning probabilistic intensity functions (i.e. transition rates) to discrete demographic events (e.g. births, deaths). This type of model, also known as a jump process, can be simulated with Gillespie's stochastic simulation algorithm (SSA) to produce samples from a continuous-time stochastic process (Gillespie 1977, Black and McKane 2012). The stochastic analogue of model 1 has the following six discrete events:

$$R \text{ birth} : R \xrightarrow{Rb_1} R + 1 \quad (3)$$

$$R \text{ death} : R \xrightarrow{R(m_1 + m_2 R) + (1 - e_1) a_1 R N_1 + (1 - e_2) a_2 R N_2} R - 1$$

$$R \text{ death, } N_1 \text{ birth} : R, N_1 \xrightarrow{e_1 a_1 R N_1} R - 1, N_1 + 1$$

$$R \text{ death, } N_2 \text{ birth} : R, N_2 \xrightarrow{e_2 a_2 R N_2} R - 1, N_2 + 1$$

$$N_1 \text{ death} : N_1 \xrightarrow{d_1 N_1} N_1 - 1$$

$$N_2 \text{ death} : N_2 \xrightarrow{d_2 N_2} N_2 - 1$$

with terms b_1 , m_1 , m_2 representing explicit birth (density-independent) and death rates (density-independent and density-dependent), which replace terms r and K . Each equation above an arrow represents the probability that a transition occurs in the community. For example, the first line (R birth) represents the birth of a resource (i.e. the population size of R increases by 1) and occurs with probability equal to Rb_1 . The expected value of model 3 matches model 1 for large population sizes, except for differences arising from lattice effects (due to discrete individuals; Henson et al. 2001) and non-linear averaging (Jensen 1906, Ruel and Ayres 1999, Chesson et al. 2005, Inouye 2005). Further details on developing the stochastic model are given in Supplementary material Appendix 2.

We simulated model 3 using the SSA across 11 different sets of parameter values, beginning with the neutral case (i.e. both consumers had identical demographic parameters), then modifying the death rate of consumer 2 so that its R^* value dropped in 5% increments, equivalent to fitness inequality increments of approximately 0.1 (Eq. 2). Parameter values for these simulations (Table 1) were chosen such that: 1) they produced dynamics broadly similar to those observed in our experimental system; 2) quasi-equilibria for consumers were near what we observed in our experimental single-consumer cultures; 3) the quasi-equilibrium value of the resource was higher than the consumer equilibria by at least two orders

Table 1. Parameter values for the neutral simulations of the stochastic consumer–resource model. The consumer attack rates, a_i , and resource death rate, m_2 , were scaled by population size to ensure simulations started in the same area of state space (with respect to density). Non-neutral simulations also used these parameter values, lowering only the death rate of consumer 2 in increments of 0.01 (making consumer 2 an increasingly superior competitor).

Model parameters	Description	Values
b_1	resource birth rate	1.2
m_1	resource death rate (density-independent)	0.2
m_2 (small)	resource death rate (density-dependent)	1/10 000
m_2 (large)		1/100 000
a_1 (small)	attack rate, consumer 1	0.04
a_1 (large)		0.004
a_2 (small)	attack rate, consumer 2	0.04
a_2 (large)		0.004
e_1, e_2	efficiencies, consumers 1 and 2	0.02
d_1, d_2	death rate, consumer 1 and 2	0.2

of magnitude; and 4) consumer death rates were high. In preliminary testing, we found that stochastic simulations of consumer–resource models with a highly abundant resource such as bacteria (with densities as high as 10^8 cells per ml) were computationally impractical using our methods. Thus, point 3) is a compromise to speed computation by assuming that the bacterial resource grows, dies, and is consumed in multiples of individuals.

For each set of parameter values, we simulated model 3 10 000 times using the Gillespie algorithm (Gillespie 1977, Black and McKane 2012) for two different initial population sizes (and maximum population sizes via resource carrying capacity), which differed by an order of magnitude in terms of absolute abundance: small ($R(0) = 1000$; $N_1(0) = N_2(0) = 5$), and large ($R(0) = 10\ 000$; $N_1(0) = N_2(0) = 50$). Simulations were run to $t = 1000$, at which point competitive exclusion had been observed in most of the neutral simulations, where the two consumer species had identical R^* values (competitive exclusion happened earlier as fitness inequality increased).

To ensure that simulations of small and large population sizes differed only with respect to the strength of demographic stochasticity, we scaled all density-dependent parameters so that simulations began in the same area of state space (with respect to density) and so that the different population sizes experienced the same degree of density-dependence throughout (Nisbet et al. 2016). Specifically, the density-dependent death rate of the resource, m_2 and the attack rates, a_i , which represent the proportion of the total habitat area subject to attack, were reduced by a factor of 10 for the large population sizes. R code for the simulations is available on the Dryad repository.

Analysis of simulations

We quantified the proportion over time of competitive exclusions of the weaker consumer by the superior consumer (i.e. simulations where the superior consumer outlasts the weaker consumer) across the different fitness inequalities and population sizes. Further, we assessed how population size affected mean densities over time. To do so, for each parameter

combination of the stochastic model, we calculated the mean density (and its 95% confidence interval, as in the experiment) of the 10 000 replicate simulations for each discrete time point. As for the experimental data, we then assessed whether these intervals overlapped.

Data deposition

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.c3bm2j9>> (Legault et al. 2019).

Results

Competitive exclusion in the experimental microcosms

Based on the competitive hierarchy described above, we expected the *aurelia*–*caudatum* pairing to be characterized by a small fitness inequality favoring the exclusion of *Paramecium aurelia* by *P. caudatum*. These assumptions agreed with our experimental findings: *P. aurelia* was the weakest competitor in the *aurelia*–*caudatum* pairing and was excluded by *P. caudatum* in 10 of 12 replicates of the large jar size (Fig. 1a). The exclusion frequency in the medium jars was similarly high; *P. aurelia* was outcompeted in 11 of 12 replicates. In the small jars, however, where the impact of demographic stochasticity was strongest, the exclusion frequency was half that of the other sizes, with *P. aurelia* excluded in only 5 of 12 replicates. Consistent with these qualitative differences, there was a statistically significant difference between exclusion frequency in small jars versus those in the large or medium jars (chance of being excluded, compared to large [logit scale]: estimate = -1.945 , standard error = 0.971, p-value = 0.045).

The weakest competitor in the *aurelia*–*philodina* pairing, *P. aurelia*, was excluded in all replicates across jar sizes (Fig. 1b). Similarly, for the *philodina*–*caudatum* pairing, *P. caudatum* was excluded in all replicates and population sizes (Fig. 1c). As there were no differences in the ultimate exclusion outcomes across jar sizes for the *aurelia*–*philodina*

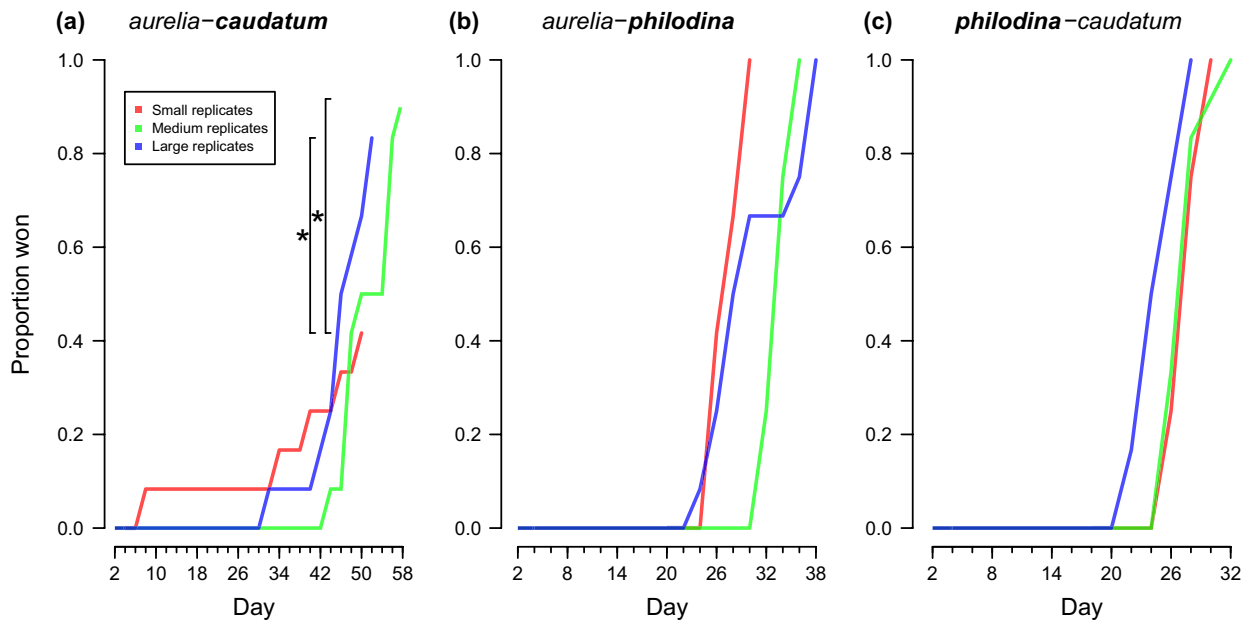


Figure 1. Proportion over time of experimental jars in which the superior competitor excluded the weaker competitor. *Paramecium aurelia* was the weakest competitor in the (a) *aurelia*–*caudatum* and (b) *aurelia*–*philodina* pairings, while *Paramecium caudatum* was the weakest competitor in the (c) *philodina*–*caudatum* pairing. Different colored lines represent the proportion surviving (out of 12 replicates) in different jar sizes (red = 1 ml habitat; green = 10 ml habitat; blue = 80 ml habitat). Lines end on the last day exclusion was observed for a treatment (x-axis), indicating the final proportion of jars where the superior competitor won (y-axis). Bars and asterisks in (a) indicate significant differences between jar sizes in the final proportion of exclusions (generalized linear model, binomial response variable).

and *philodina*–*caudatum* pairings, statistical analysis was not necessary for these data.

Competitive exclusion in the stochastic simulations

When the fitness inequality was 0 (i.e. consumers had identical R^* s), simulations with small or large population sizes had nearly identical outcomes in terms of the identity of the ‘winning’ consumer. For the large population sizes (Fig. 2, solid lines), by $t = 1000$, consumer 1 had excluded consumer 2 in 47.80% of simulations and consumer 2 had excluded consumer 1 in 48.16% of simulations. Of the remaining simulations, 325 (3.25%) exhibited joint exclusion/extinction, meaning both species had gone extinct between time steps (while the model was simulated in continuous-time, we observed the system only at discrete times), and 79 (0.79%) still had both species (i.e. transient coexistence). For the small population sizes (Fig. 2, dashed lines), the percentages were quite similar: consumer 2 was excluded in 48.55% of simulations, consumer 1 was excluded in 47.95% of simulations, and both were excluded in 3.5% of simulations (no transient coexistence was observed). However, exclusion occurred much earlier for small population sizes (Fig. 2).

When fitness inequalities were non-zero (i.e. non-neutral), competitive outcomes for small and large populations differed substantially in two ways: 1) as in the neutral case, exclusion occurred earlier for small population sizes across all fitness inequalities (Fig. 2); and 2) the proportion of simulations in which the ‘superior’ consumer won was consistently lower

for small population sizes (Fig. 2) across all fitness inequalities. In the deterministic version of the consumer–resource model, any non-zero fitness inequalities between consumers will always lead to the exclusion of the weaker competitor (i.e. consumer with the largest R^*). In general, this is what was observed for non-neutral simulations with large population sizes, where the proportion of simulations won by the superior competitor rapidly approach 1.0 as fitness inequalities increased. In contrast, stochasticity in simulations with small population sizes led to many simulations where the weaker consumer excluded (i.e. outlasted) its ‘superior’ rival (Fig. 2). As fitness inequalities increased from 0, the proportion of small simulations where the superior consumer won increased only gradually, well below the increases seen for the large simulations. Only when fitness inequalities were very large (e.g. consumer 2 had an R^* 50% lower than consumer 1, corresponding to a fitness inequality of 1.5) were these proportions similar to those of the large populations (Fig. 2). Such differences in the proportion of simulations won by the superior consumer were not due to differences in the timing of exclusion, since by $t = 1000$, 99% of all simulations had experienced exclusion.

Mean densities in the experimental microcosms

For the *aurelia*–*caudatum* pairing, where the fitness inequality between competitors was small, both species had consistently lower densities in small jars relative to large jars (Fig. 3a–b). In other words, when demographic stochasticity

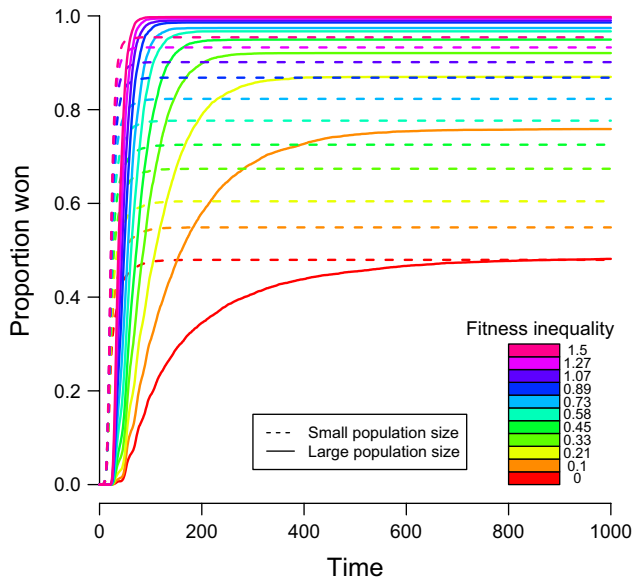


Figure 2. Proportion over time of the stochastic simulations in which the superior consumer (consumer 2) excluded the weaker consumer (consumer 1). Shown are the proportions for 11 different fitness inequalities, ranging from the neutral case (fitness inequality = 0, red) to one where consumer 1 has twice the R^* as consumer 2 (fitness inequality = 1.5; purple). Dotted lines show the results for simulations with small population sizes and solid lines show the results for simulations with large population sizes. Other possible outcomes were exclusion of the superior competitor (majority of cases), transient coexistence, or joint exclusion (no winner of competition). See Table 1 for the parameter values used in the neutral simulations (i.e. when consumers had the same fitness).

was strong, both consumers had lower mean densities. When fitness inequalities were larger, as in the *aurelia-philodina* and *philodina-caudatum* pairings, densities of the weaker competitors were also lower in small jars compared to large jars (Fig. 3c, e). However, jar size appeared to have less severe effects on the mean densities of the superior competitors in the *aurelia-philodina* and *philodina-caudatum* pairings (Fig. 3d, f). Densities in the medium-sized jars (not shown in Fig. 3 for clarity) were generally between those of the large and small jars, or were similar to the large jars (Supplementary material Appendix 1 Fig. A3).

Mean densities in the stochastic simulations

Demographic stochasticity also affected the mean densities of consumers in the simulations. In general, early in the time series the mean densities of both consumers were similar for small and large population sizes (Fig. 4). Over the long term, however, mean densities were generally lower for small population sizes (95% confidence intervals for expected values generally did not overlap). Furthermore, clear qualitative differences in overall dynamics emerged between small and large simulations, as evidenced by changes in the amplitude and periodicity of mean densities. These results were consistent across all 11 fitness inequalities.

Discussion

We explored the effects of demographic stochasticity on competition using experimental communities of microzooplankton and simulations of continuous-time stochastic models, demonstrating that stochasticity (here driven by absolute abundances) can alter competitive outcomes and other aspects of community dynamics in non-neutral communities. In particular, in both simulated and microcosm communities, demographic stochasticity reduced the exclusion frequency of weaker competitors, led to earlier exclusions, and generally lowered mean densities over time.

One of our key findings, that population size can alter competitive outcomes in microzooplankton communities, provides empirical support for existing ecological theory concerning the effects of demographic stochasticity in simple, non-neutral communities. Orrock and Fletcher (2005) and Pedruski et al. (2015) both used discrete-time models of consumer–resource dynamics and an approximation of demographic stochasticity to show that it can allow weaker competitors to out-compete superior competitors when niche differences were small, but not when they were large. Similarly, Okuyama (2015) used continuous-time models of consumer–resource dynamics to show that demographic stochasticity can lead to competitive outcomes not predicted by the deterministic model. Our simulation results extend such work by examining the effects of demographic stochasticity on competitive outcomes across different absolute abundances and fitness differences. In particular, across 11 sets of stochastic simulations, we found that the identity of the winning species was more variable for small population sizes compared to large population sizes. Specifically, the proportion of simulations won by the superior competitor was generally lower and occurred faster in small populations compared to large populations across a wide range of fitness inequalities. Only when there were large fitness inequalities were exclusion frequencies and times similar between population sizes. Furthermore, our experimental findings demonstrate how the effects of demographic stochasticity can manifest in real, non-neutral communities: we found that when fitness differences were small, a weaker competitor (*Paramecium aurelia*) was able to exclude its superior rival (*P. caudatum*) more frequently in small jars compared to populations in larger jars with otherwise comparable environmental conditions. However, when fitness differences were large, as they were in the other species pairings, jar size had no discernible effect on competitive outcomes; that is, the species with the highest competitive ability always excluded the other. This finding is broadly consistent with Fox (2002), which found that the ' R^* rule' was not an accurate predictor of competitive dominance in protist microcosms when the gaps between species' R^* s were smaller. Thus our experimental results are consistent with previous theoretical and empirical studies, providing support for the notion that absolute abundance is an important consideration when assessing the relative importance of

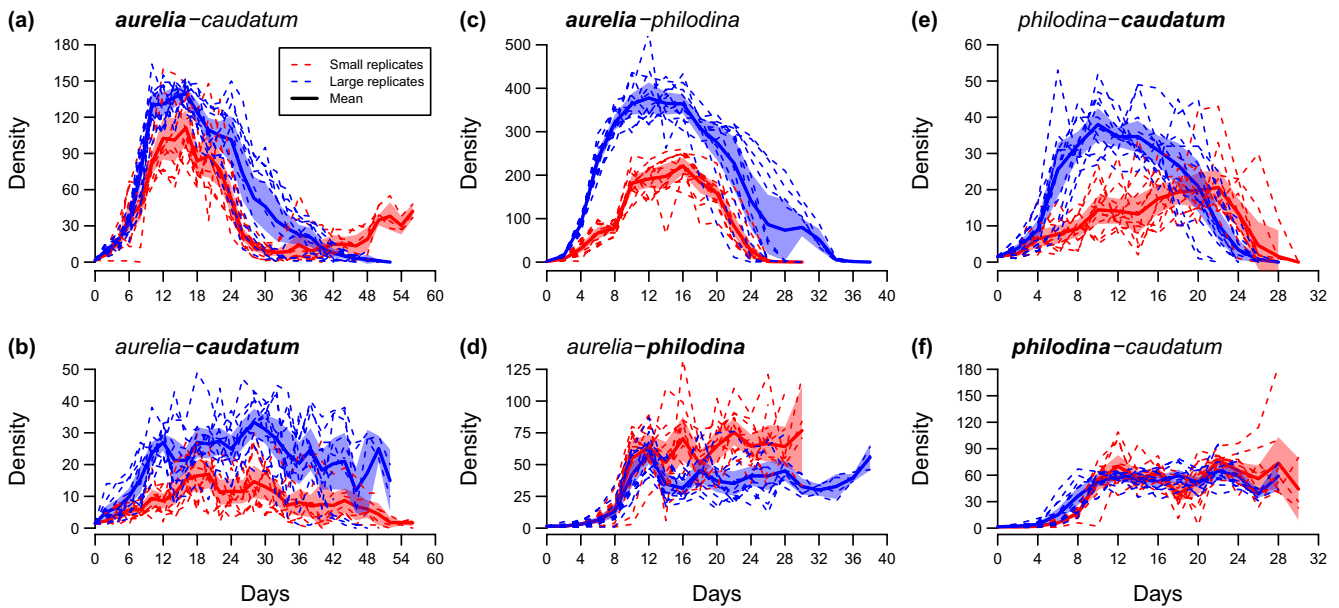


Figure 3. Densities over time in the experimental microcosms. Shown are the densities of individual replicates and their means for the weaker competitors (top row) and superior competitors (bottom row) in each of the three competitive pairings (*aurelia*–*caudatum*, column 1; *aurelia*–*philodina*, column 2; *philodina*–*caudatum*, column 3). Bold names represent the species being plotted in each panel. Also plotted are the 95% confidence intervals for the mean densities (shaded areas). Due to replicates being removed once exclusion had occurred, some intervals are especially large or missing at different time points. For clarity, only small (red) and large (blue) jars are shown here (see Supplementary material Appendix 1 for medium jars). Note the different scales on both axes for each panel.

niche versus stochastic processes in communities (Adler et al. 2007, Gravel et al. 2011, Vellend et al. 2014).

In addition to observing differences in competitive outcomes, we found that jar size affected the densities over time of consumers in both our experimental jars and in our simulations. In the *aurelia*–*caudatum* pairing, where fitness differences were small (*P. aurelia* and *P. caudatum*), each species had lower mean densities in small jars compared to large jars. When fitness differences were larger, as in the *philodina*–*caudatum* and *aurelia*–*philodina* pairings, only the densities of the weaker competitor were reduced in the presence of strong demographic stochasticity. Similarly, in the simulations, initial densities in the small populations were lower over the long-term than those in the large populations for both consumers.

How does small population size interact with demographic stochasticity to affect competitive outcomes and mean densities in our experimental and simulated communities? One answer is that small populations have fewer individuals and therefore fewer stochastic events governing abundances over time. This can lead to large deviations from expected outcomes such as who wins competition in essentially the same way that a small number of coin flips will not necessarily produce the expected outcome of 50% tails. When processes are non-linear, such added variation can also affect mean outcomes, including densities over time. Large populations are also capable of reaching finer fractional densities (e.g. 0.001 individuals ml⁻¹) whereas small populations are more coarsely discretized, so lattice effects may also lead to larger deviations from expected densities (Henson et al. 2001). Finally,

stochastic extinctions, which have higher probability in smaller populations, will also lower mean densities compared to deterministic dynamics.

We likely did not observe the theoretically predicted long-term increases/decreases in the densities of weaker/superior consumers in our experimental system for two reasons: 1) our experiment did not persist long enough; and 2) our species did not experience the same degree of demographic stochasticity as in our simulations. Regarding 2), this can be seen by comparing Fig. 3, 4, which highlight the often large differences in abundance between the two species competing in the experiment, compared to the mostly similar consumer abundances in the simulations. For instance, in the *aurelia*–*philodina* pairing, *P. aurelia* had approximately four-fold higher absolute abundances over time than its competitor *P. caudatum*. We intentionally chose to simulate consumers with similar absolute abundances as it meant that both consumers would experience similar degrees of demographic stochasticity for each simulation treatment. However, this choice also ignored the very real possibility that some consumers may have lower mean or maximum densities than their competitors (e.g. due to resource requirements), and therefore experience demographic stochasticity differently across habitat sizes. This was likely the case for some of our experimental jars.

Founder effects could have explained some of the differences we observed in competitive outcomes across population sizes in our experiment; however, this effect was likely negligible. If founder effects were important, we would expect to see large differences over time between replicates of the small jars compared to replicates of larger jars due to the

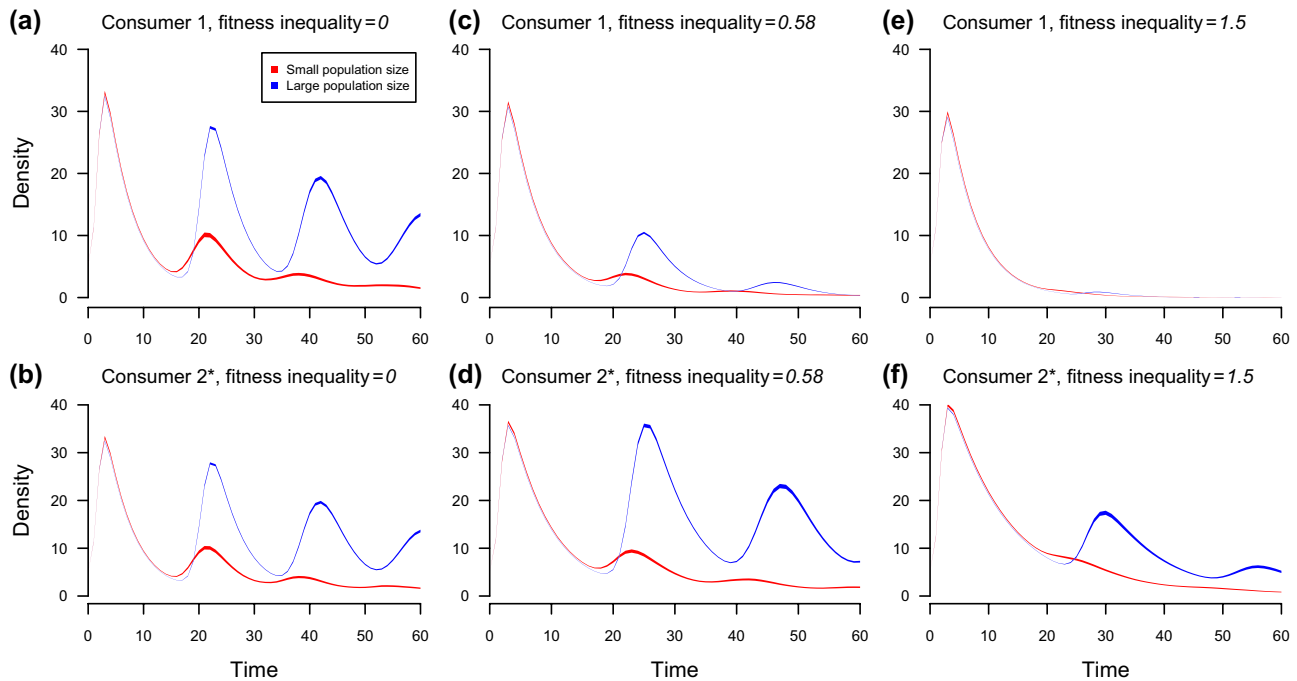


Figure 4. Mean densities over time of consumers from the stochastic simulations (i.e. expected values of the stochastic model). Shown are the 95% confidence intervals of the mean densities over time of the weaker competitors (top row) and superior competitors (bottom row) for increasing fitness inequalities from 0 to 1.5 for simulations with large (red) and small (blue) population sizes (confidence intervals may be small enough to be indiscernible).

stronger influence of genetic drift in the small jars over and above the effects of demographic stochasticity (Dobzhansky and Pavlovsky 1957). However, the coefficients of variation across replicates were not consistently higher for the small jars (Supplementary material Appendix 3). Moreover, the stock cultures used for our experiment had spent more than 1.5 years at conditions similar to those used in the experiment, representing thousands of generations in a constant environment. As a result, the standing genetic diversity of the protists used in our competitive trials was likely to be low, meaning that different jar sizes probably had similar starting diversities and genetic composition.

Previous experiments have shown that the species considered here strongly compete (Gause 1934, Vandermeer 1969, Cadotte et al. 2006, DeLong and Vasseur 2012). As discussed in Supplementary material Appendix 1, comparison of the two-consumer laboratory cultures with single-consumer cultures show that dynamics are clearly affected by the presence of a second consumer (in particular, lowering mean densities), thus confirming that the species strongly compete. The biological mechanisms of competition between our three focal species are not precisely known, although it is likely that they experience both direct (e.g. allelopathy) and indirect interactions (e.g. resource competition).

We were unable to completely disentangle the effects of demographic stochasticity from potential (deterministic) habitat-size artifacts in the experiment, meaning that our results should be considered with some caution. Possible habitat-size artifacts include wall effects, behavioral responses to

habitat size, differences in spatial heterogeneity, and chemical effects related to surface area to volume ratios (Englund and Cooper 2003), many of which could apply to our laboratory system. Unfortunately, controlling for these artifacts can be extremely difficult if not impossible in any study using habitat size manipulations to understand stochasticity. In particular, single-consumer cultures of different habitat sizes cannot provide controls for all habitat-size artifacts. For our system, this is revealed by single-consumer simulations with small and large population sizes, which show large differences in temporal dynamics and densities between population sizes (Supplementary material Appendix 4). Since demographic stochasticity alone alters density and dynamics in single-consumer cultures, different jar sizes with single consumers cannot provide controls for deterministic effects of jar size. One possible way forward would be to fit stochastic models to the experimental data for both single- and two-species communities. In principle, a fitted stochastic model could account for variation arising from demographic stochasticity alone and make it possible to identify additional variation in the data that could be attributed to jar size effects. However, we were not able to fit models that were good enough to be used for inference (Supplementary material Appendix 1). Importantly, while this caveat applies to our experimental results, it does not apply to our simulation results where, by design, community size was not confounded with any other factors and results were qualitatively consistent with the laboratory experiment. Practical solutions to habitat-size artifacts might also be possible, but it is difficult to envision manipulations

free of any size-related confounds. For instance, using fine-meshed compartments of varying sizes in identically sized containers would avoid effects related to the edges of containers, but could introduce refugia for bacterial resources outside the mesh.

We also consider possible errors that could have been introduced through differences in sampling intensity and conclude that these are unlikely to be important. As described in the methods, we sampled a larger fraction of the volume of small jars (30%) compared to medium or large jars (10%). This difference in sampling intensity allowed us to include more experimental replicates but it could potentially influence estimates of exclusion frequency. However, the results suggest these sampling effects, if present at all, were minimal. All competitive pairings were subject to the same sampling scheme and so if sampling effects were driving the results, we would expect to see similar differences in exclusion outcome across all species pairings. On the contrary, we only observed differences in exclusion in the *aurelia*–*caudatum* pairing. Second, all *P. aurelia* exclusions observed in the medium and large *aurelia*–*caudatum* jars occurred after long, nearly monotonic declines in population densities (Fig. 3a blue lines) and generally when its competitor, *P. caudatum*, had densities at least an order of magnitude greater (Supplementary material Appendix 3). Following this decline, there were also nine cases where our initial samples of medium or large jars on a given day suggested exclusion (five medium, four large) but where we then found the missing species in a subsequent 10% sample. In all nine cases, *P. aurelia* densities never subsequently increased. Therefore, it is likely that any differences in sampling intensity would have affected only estimates of the timing of exclusion and not its outcome. Finally, our consumer–resource simulations could not be affected by sampling effects (since complete censuses were recorded) and they also showed clear differences in competitive outcomes among population sizes due to demographic stochasticity (Fig. 2).

As argued by Vellend (2010, 2016), the effects of demographic stochasticity (i.e. ‘ecological drift’) on community dynamics are analogous to the effects of genetic drift on evolutionary dynamics. This is because both ecological and genetic drift involve stochastic processes: for ecological drift these are the effectively random demographic processes (birth, death, migration, maturation) that affect population sizes; for genetic drift, these are factors such as the random sampling of alleles every generation. As far back as Wright (1931), evolutionary biologists have recognized that at small population sizes, genetic drift can interfere with selection, for instance by preventing a beneficial allele from reaching fixation. There is now a vast literature concerning the effects of genetic drift and population size on fixation probabilities (Patwa and Wahl 2008), as well as numerous empirical studies demonstrating that small population sizes can lead to evolutionary outcomes not predicted by deterministic models of evolution (Dobzhansky and Pavlovsky 1957, Weber 1990, Lynch and Conery 2003, Paland and Schmid 2003, Petit and Barbadilla 2009). Studies of the consequences of ecological drift are less

common, in part because the processes involved are complex: there are typically many stochastic processes occurring at the population- and community-level (Melbourne and Hastings 2008) and they are often non-independent. Moreover, ecologists are generally less willing to make the simplifying assumptions common to evolutionary studies of genetic drift, such as the constant population size required by Kimura’s (1957) equations for the probability of fixation of beneficial alleles. Nevertheless, such assumptions may be reasonable for some ecological systems (Hubbell 2001), and additional synthesis between evolutionary and ecological conceptions of drift may be possible.

Demographic stochasticity is ubiquitous in natural systems and represents a significant source of intraspecific variation in populations and communities. Recognizing the potential role for such stochasticity in non-neutral communities is important, as it can produce outcomes not predicted by common, deterministic models of competition and other processes. Future work should focus on how to better characterize and quantify the impacts of demographic stochasticity in natural, non-neutral communities and in more realistic continuous-time descriptions of such systems. Even in a laboratory-microcosm system such as ours with tight experimental control, this is not easy. For instance, to definitively isolate the effect of demographic stochasticity in real communities from other possible confounders, such as edge effects or jar effects, it is necessary to fit continuous-time stochastic models to the relevant data, a step that must be preceded by the development and testing of appropriate stochastic population models (Melbourne and Hastings 2008). However, given the potential for demographic stochasticity to produce outcomes that diverge from deterministic expectations, such work may be necessary if we are to better predict how non-neutral communities assemble and change with time.

Acknowledgements – The manuscript was much improved thanks to comments by C. M. Tucker.

Author contributions and funding – GL and JWF contributed to the experimental design. The experimental work was conducted by GL while he was a graduate student at the University of Calgary, supported by an NSERC Discovery grant to JWF. GL conducted the stochastic simulation work with contributions from BAM, who was supported by a grant from the National Science Foundation (DEB 1457660). GL, JWF and BAM contributed to the writing of the manuscript.

References

- Adler, P. B. et al. 2007. A niche for neutrality. – *Ecol. Lett.* 10: 95–104.
- Alexander, H. M. et al. 2012. Metapopulations and metacommunities: combining spatial and temporal perspectives in plant ecology. – *J. Ecol.* 100: 88–103.
- Bartlett, M. 1960. Stochastic population models in ecology and epidemiology. – Methuen.
- Bell, G. 2000. The distribution of abundance in neutral communities. – *Am. Nat.* 155: 606–617.

- Belovsky, G. E. et al. 1999. Experimental studies of extinction dynamics. – *Science* 286: 1175–1177.
- Black, A. J. and McKane, A. J. 2012. Stochastic formulation of ecological models and their applications. – *Trends Ecol. Evol.* 27: 337–345.
- Bolnick, D. I. et al. 2011. Why intraspecific trait variation matters in community ecology. – *Trends Ecol. Evol.* 26: 183–192.
- Burkey, T. V. 1997. Metapopulation extinction in fragmented landscapes: using bacteria and protozoa communities as model ecosystems. – *Am. Nat.* 150: 568–591.
- Cadotte, M. W. et al. 2006. On testing the competition–colonization tradeoff in multispecies assemblage. – *Am. Nat.* 168: 704–709.
- Capitán, J. et al. 2015. How similar can co-occurring species be in the presence of competition and ecological drift? – *J. R. Soc. Interface* 12: 20150604.
- Chase, J. M. and Myers, J. A. 2011. Disentangling the importance of ecological niches from stochastic processes across scales. – *Phil. Trans. R. Soc. B* 366: 2351–2363.
- Chave, J. 2004. Neutral theory and community ecology. – *Ecol. Lett.* 7: 241–253.
- Chesson, P. 2000. Mechanisms of maintenance of species diversity. – *Annu. Rev. Ecol. Syst.* 31: 343–366.
- Chesson, P. et al. 2005. Scale transition theory for understanding mechanisms in metacommunities. – In: Leibold, M. A. and Holt, R. D. (eds), *Metacommunities: spatial dynamics and ecological communities*. Univ. of Chicago Press, chap. 12, pp. 279–306.
- DeLong, J. P. and Vasseur, D. A. 2012. Size-density scaling in protists and the links between consumer–resource interaction parameters. – *J. Anim. Ecol.* 81: 1193–1201.
- Dickerson, J. E. and Robinson, J. V. 1985. Microcosms as islands: a test of the MacArthur–Wilson equilibrium theory. – *Ecology* 66: 966–980.
- Dobzhansky, T. and Pavlovsky, O. 1957. An experimental study of interaction between genetic drift and natural selection. – *Evolution* 11: 311–319.
- Engen, S. et al. 2005. Extinction in relation to demographic and environmental stochasticity in age-structured models. – *Math. Biosci.* 195: 210–227.
- Engen, S. et al. 2017. Neutral or non-neutral communities: temporal dynamics provide the answer. – *Oikos* 126: 318–331.
- Englund, G. and Cooper, S. D. 2003. Scale effects and extrapolation in ecological experiments. – *Adv. Ecol. Res.* 33: 161–213.
- Fox, J. W. 2002. Testing a simple rule for dominance in resource competition. – *Am. Nat.* 159: 305–319.
- Fox, J. W. and Smith, D. C. 1997. Variable outcomes of protist–rotifer competition in laboratory microcosms. – *Oikos* 79: 489–495.
- Fukami, T. 2004. Assembly history interacts with ecosystem size to influence species diversity. – *Ecology* 85: 3234–3242.
- Gause, G. 1934. *The struggle for existence*. – Williams and Wilkins Company.
- Gilbert, B. and Levine, J. M. 2017. Ecological drift and the distribution of species diversity. – *Proc. R. Soc. B* 284: 20170507.
- Gillespie, D. T. 1977. Exact stochastic simulation of coupled chemical reactions. – *J. Phys. Chem.* 81: 2340–2361.
- Gravel, D. et al. 2011. Species coexistence in a variable world. – *Ecol. Lett.* 14: 828–839.
- Griffen, B. D. and Drake, J. M. 2008. A review of extinction in experimental populations. – *J. Anim. Ecol.* 77: 1274–1287.
- Henson, S. M. et al. 2001. Lattice effects observed in chaotic dynamics of experimental populations. – *Science* 294: 602–605.
- Hubbell, S. P. 2001. *The unified neutral theory of biodiversity and biogeography*. – Princeton Univ. Press.
- Inouye, B. D. 2005. The importance of the variance around the mean effect size of ecological processes: comment. – *Ecology* 86: 262–265.
- Jensen, J. 1906. Sur les fonctions convexes et les inégalités entre les valeurs moyennes. – *Acta Math.* 3: 175–193.
- Kimura, M. 1957. Some problems of stochastic processes in genetics. – *Ann. Math. Stat.* 28: 882–901.
- Kramer, A. M. and Drake, J. M. 2014. Time to competitive exclusion. – *Ecosphere* 5: 1–16.
- Kurtz, T. G. 1970. Solutions of ordinary differential equations as limits of pure jump markov processes. – *J. Appl. Probabil.* 7: 49–58.
- Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. – *Am. Nat.* 142: 911–927.
- Legault, G. et al. 2019. Data from: demographic stochasticity alters expected outcomes in experimental and simulated non-neutral communities. – Dryad Digital Repository, <<http://dx.doi.org/10.5061/dryad.c3bm2j9>>.
- Lotka, A. 1925. *Elements of physical biology*. – Williams and Wilkins Company.
- Lynch, M. and Conery, J. S. 2003. The origins of genome complexity. – *Science* 302: 1401–1404.
- MacArthur, R. 1970. Species packing and competitive equilibrium for many species. – *Theor. Popul. Biol.* 1: 1–11.
- Matis, J. and Kiffe, T. 2000. *Stochastic population models: a compartmental perspective*. – Springer.
- May, R. 1973. *Stability and complexity in model ecosystems*. – Princeton Univ. Press.
- Melbourne, B. A. 2012. Demographic stochasticity. – In: *Encyclopedia of theoretical ecology*. Univ. of California Press, p. 848.
- Melbourne, B. A. and Hastings, A. 2008. Extinction risk depends strongly on factors contributing to stochasticity. – *Nature* 454: 100–103.
- Mertz, D. B. et al. 1976. An experimental analysis of competitive indeterminacy in *Tribolium*. – *Proc. Natl Acad. Sci. USA* 73: 1368–1372.
- Nisbet, R. M. et al. 2016. Integrating ecological insight derived from individual-based simulations and physiologically structured population models. – *Ecol. Model.* 326: 101–112.
- Okuyama, T. 2015. Demographic stochasticity alters the outcome of exploitation competition. – *J. Theor. Biol.* 365: 347–351.
- Orrock, J. L. and Fletcher, R. J. 2005. Changes in community size affect the outcome of competition. – *Am. Nat.* 166: 107–111.
- Orrock, J. L. and Watling, J. I. 2010. Local community size mediates ecological drift and competition in metacommunities. – *Proc. R. Soc. B* 277: 2185–2191.
- Ovaskainen, O. and Meerson, B. 2010. Stochastic models of population extinction. – *Trends Ecol. Evol.* 25: 643–652.
- Paland, S. and Schmid, B. 2003. Population size and the nature of genetic load in *Gentianella germanica*. – *Evolution* 57: 2242–2251.
- Park, T. 1954. Experimental studies of interspecies competition II. Temperature, humidity and competition in two species of *tribolium*. – *Physiol. Zool.* 27: 177–238.
- Patwa, Z. and Wahl, L. M. 2008. The fixation probability of beneficial mutations. – *J. R. Soc. Interface* 5: 1279–1289.

- Pedruski, M. T. et al. 2015. Predicting the outcome of competition when fitness inequality is variable. – *R. Soc. Open Sci.* 2: 150274.
- Petit, N. and Barbadilla, A. 2009. Selection efficiency and effective population size in *Drosophila* species. – *J. Evol. Biol.* 22: 515–526.
- Renshaw, E. 1991. Modelling biological populations in space and time. – Cambridge Univ. Press.
- Rosindell, J. et al. 2012. The case for ecological neutral theory. – *Trends Ecol. Evol.* 27: 203–208.
- Ruel, J. J. and Ayres, M. P. 1999. Jensen's inequality predicts effects of environmental variation. – *Trends Ecol. Evol.* 14: 361–366.
- Shaffer, M. L. 1981. Minimum population sizes for species conservation. – *BioScience* 31: 131–134.
- Siepielski, A. M. et al. 2010. Experimental evidence for neutral community dynamics governing an insect assemblage. – *Ecology* 91: 847–857.
- Spencer, M. and Warren, P. H. 1996. The effects of habitat size and productivity on food web structure in small aquatic microcosms. – *Oikos* 75: 419–430.
- Svensson, E. I. et al. 2018. Frequency dependence and ecological drift shape existence of species with similar niches. – *Am. Nat.* 191: 691–703.
- Tilman, D. 1982. Resource competition and community structure. – Princeton Univ. Press.
- Tilman, D. 2004. Niche tradeoffs, neutrality and community structure: a stochastic theory of resource competition, invasion and community assembly. – *Proc. Natl Acad. Sci. USA* 101: 10854–10861.
- Vandermeer, J. H. 1969. The competitive structure of communities: an experimental approach with Protozoa. – *Ecology* 50: 362–371.
- Vellend, M. 2010. Conceptual synthesis in community ecology. – *Q. Rev. Biol.* 85: 183–206.
- Vellend, M. 2016. The theory of ecological communities. – Princeton Univ. Press.
- Vellend, M. et al. 2014. Assessing the relative importance of neutral stochasticity in ecological communities. – *Oikos* 123: 1420–1430.
- Vindenes, Y. et al. 2008. Individual heterogeneity in vital parameters and demographic stochasticity. – *Am. Nat.* 171: 455–467.
- Volkov, I. et al. 2003. Neutral theory and relative species abundance in ecology. – *Nature* 424: 1035–1037.
- Volterra, V. 1926. Variazioni e fluttuazioni del numero d'individui in specie animali conviventi. – *Mem. Accad. Lincei* 2: 31–113.
- Wang, X. et al. 2016. Stochastic dilution effects weaken deterministic effects of niche-based processes in species rich forests. – *Ecology* 97: 347–360.
- Weber, K. E. 1990. Increased selection response in larger populations. I. Selection for wing-tip height in *Drosophila melanogaster* at three population sizes. – *Genetics* 125: 579–584.
- Wright, S. 1931. Evolution in Mendelian populations. – *Genetics* 16: 97–159.

Supplementary material (available online as Appendix oik-06028 at <www.oikosjournal.org/appendix/oik-06028>). Appendix 1–4.